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SANTA CRUZ BIOTECHNOLOGY, INC.

DNase I siRNA (h): sc-41505

BACKGROUND

Internucleosomal DNA fragmentation following the activation of endonucleases is the common end point of apoptosis. DNase I, a Ca^{2+}/Mg^{2+}-dependent endonuclease ubiquitously expressed in mammalian tissues, has been implicated to mediate internucleosomal DNA degradation in human cells undergoing apoptosis. DNase I is highly polymorphic, and at least six alleles of DNase I are known. DNase II, the ubiquitously expressed acidic deoxyribonuclease, acts downstream of caspase activation and may also induce DNA digestion during apoptosis. DNase I cleaves DNA to 5'-phosphodinucleotide and 5'-phosphooligonucleotide end-products, whereas DNase II cleaves DNA to 3'-phosphomononucleotide and 3'-phosphooligonucleotide end-products. The mechanism by which DNase II cuts DNA is similar to DNase I, which produces nicks rather than double-strand cuts. DNase II is usually present in cytoplasm of epithelial cells, but it appears concentrated in the nuclei of lens fibers. In contrast, DNase I is always concentrated in nuclei of epithelial and fiber cells. The gene encoding DNase II maps to human chromosome 19.

REFERENCES

1. Torriglia, A., et al. 1995. Involvement of DNase II in nuclear degeneration during lens cell differentiation. J. Biol. Chem. 270: 28579-28585.
2. Yasuda, T., et al. 1998. Molecular cloning of the cDNA encoding human deoxyribonuclease II. J. Biol. Chem. 273: 2610-2616.
3. Krieser, R.J. and Eastman, A. 1998. The cloning and expression of human deoxyribonuclease II. A possible role in apoptosis. J. Biol. Chem. 273: 30909-30914.
4. Baker, K.P., et al. 1998. Molecular cloning and characterization of human and murine DNase II. Gene 215: 281-289.
5. Yasuda, T., et al. 1999. A new allele, DNASE1*6, of human deoxyribonuclease I polymorphism encodes an Arg to Cys substitution responsible for its instability. Biochem. Biophys. Res. Commun. 260: 280-283.
6. Oliveri, M., et al. 2001. DNase I mediates internucleosomal DNA degradation in human cells undergoing drug-induced apoptosis. Eur. J. Immunol. 31: 743-751.

CHROMOSOMAL LOCATION

Genetic locus: DNASE1 (human) mapping to 16p13.3.

PRODUCT

DNase I siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DNase I shRNA Plasmid (h): sc-41505-SH and DNase I shRNA (h) Lentiviral Particles: sc-41505-V as alternate gene silencing products.

For independent verification of DNase I (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41505A, sc-41505B and sc-41505C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

DNase I siRNA (h) is recommended for the inhibition of DNase I expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

DNase I (B-4): sc-376207 is recommended as a control antibody for monitoring of DNase I gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DNase I gene expression knockdown using RT-PCR Primer: DNase I (h)-PR: sc-41505-PR (20 µl, 577 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.